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Antheridiogen Response In *Phanerophlebia* and Related Fern Genera

GEORGE YATSKIEVYCH
Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166, U.S.A.

Antheridiogens are naturally-occurring plant products that act pheromonally to affect sexual expression in the gametophytes of some pteridophyte species. The production of an antheridiogen was first reported by Döpp (1950), who observed that developing gametophytes of *Pteridium aquilinum* (L.) Kuhn produced a substance that caused other, newly germinated gametophytes of this species to produce antheridia precociously and abundantly, with a subsequent delay in the formation of a meristematic notch and archegonial production. Since that time, the action, expression, and biochemical structure of this type of compound have been studied in some detail (for reviews see Voeller, 1964; Näf et al., 1975).

Subsequent research has also demonstrated that three biochemically distinct general classes of antheridiogens are produced by taxonomically diverse species, best identified trivially by the capital letters A, B, and C, to denote biologically interreactive groupings (Schedlbauer and Klekowski, 1972; Haufler and Gastony, 1978). Antheridiogen A, first isolated from *P. aquilinum* (Döpp, 1950), affects gametangial development in gametophytes of some members of such diverse families as Blechnaceae, Cyatheaceae, Davalliaceae, Dennstaedtiaceae, Dicksoniaceae, Dryopteridaceae, Polypodiaceae, Pteridaceae, and Thelypteridaceae (all *sensu* Tryon and Tryon, 1982) (Näf et al., 1975; Voeller, 1964). Antheridiogen B, first demonstrated in *Anemia phyllitidis* (L.) Sw. (Näf, 1959), affects several species of Schizaeaceae. Antheridiogen C is specific to *Ceratopteris* (Pteridaceae; Schedlbauer and Klekowski 1972). Fern species tested for antheridiogen response were reviewed by Näf et al. (1975), with subsequent additions by Schedlbauer and Klekowski (1972), Haufler and Gastony (1978), and Haufler and Ranker (1985).

Antheridiogens have been implicated as functioning in nature to promote outcrossing in pteridophytes by mediating obligate cross-fertilization through ontogenetic control of gametangial formation in natural gametophyte populations (see Schneller et al., 1990, for review). Previous studies have emphasized two different approaches to the elucidation of antheridiogen function in ferns. As noted above, several studies have documented the susceptibility of gametophytes of various species to antheridiogens of "classical" producers, as well as the production of native antheridiogens by many of these same species under laboratory culture conditions. These studies have involved exogenous hormonal application by germination of spores on antheridiogen-enriched nutrient agar. Other studies have characterized populations of gametophytes in the field to demonstrate that naturally occurring gametophytes of some temperate and tropical pteridophytes do exist as unisexual plants, as would be predicted if an antheridiogen system were operative in nature (Tryon and Vitale, 1977; Schneller, 1979; Farrar and Gooch, 1975). There have not been any studies to confirm directly the action of an antheridiogen system in nature and problems of experimental design and adequate controls may be insurmountable in this regard. However, recent studies have suggested the existence of an antheridiogen system

in nature by demonstrating that naturally occurring populations in some species of *Bommeria* (Pteridaceae; Haufler and Soltis, 1984) and *Hemionitis* (Pteridaceae; Schneller et al., 1990) that are known to produce a native antheridiogen under laboratory conditions are also highly outcrossing. Other studies have also demonstrated correlations between increased genetic load and antheridiogen production (Schneller et al., 1990).

In spite of the apparent importance of antheridiogens in mediating breeding systems, there have been relatively few studies to determine the extent of antheridiogen production and susceptibility among fern species. Soltis and Soltis (1987) recently noted that less than 1% of all fern species have been tested for antheridiogen response. The present study was undertaken during general biosystematic research on the closely related genera *Cyrtomium, Phanerophlebia,* and *Polystichum* (Dryopteridaceae) to document the production of and/or susceptibility to antheridiogens of representative taxa under culture conditions. A clarification of the breeding system operating in these plants might contribute to an understanding of levels of genetic variation that exist within and between populations and, indirectly, to an understanding of systematic relationships and evolution within the group.

Polystichum is a nearly cosmopolitan genus of ca. 160 species (Tryon and Tryon, 1982). Several segregates of this large and variable genus have been described, the largest of which are the Asiatic *Cyrtomium* and the neotropical *Phanerophlebia.* In spite of the fact that several species of *Polystichum* are relatively widespread in various parts of the world, and that other species of this genus, as well as of *Cyrtomium,* are commonly cultivated for horticultural purposes, relatively few members of this complex have been examined for antheridiogen production. *Polystichum acrostichoides* (Michaux) Schott (Näf, 1956; Voeller, 1964) and *P. tsus-simense* (Hook.) J. Smith (Näf, 1969) previously have been shown susceptible to various concentrations of antheridiogen A. *Cyrtomium falcatum* (Thunb. ex L. f.) C. Presl was found to be unaffected by antheridiogen A, as was *P. tsus-simense* at lower concentrations of the hormone (Voeller, 1964). *Cyrtomium falcatum* is notable among the previously tested taxa in existing in nature primarily as an apomict, a fact not addressed by previous studies.

Material and Methods

Species surveyed in this study are listed in Table 1. Spores were collected from greenhouse-grown plants by rinsing portions of fertile fronds with distilled water and pressing between sheets of writing paper. Spore samples were sterilized and grown axenically (Haufler and Gastony, 1978). After ca. ten weeks of growth, mature gametophytes were scraped from each culture with a spatula. Agar samples were diluted with fresh agar (by weight) to yield 0.5 and 0.25 dilutions, autoclaved, repoured, and resown with appropriate sterile spores, as above. Germination and development of these latter spore samples were observed under the dissecting microscope at one week intervals for ten weeks, to record gametophyte development and ontogeny of gametangia for each species.

Pteridium aquilinum supplied antheridiogen A, with *Onoclea sensibilis* L. used as a control for the activity of this antheridiogen. Spores of each species samples (Table 1) were sown on agar containing antheridiogen A at both concentrations, to test for susceptibility to this general class of antheridiogens. Each sample species was also tested for production of a native antheridiogen, both by observing its growth on its own agar source and by observing growth of *Onoclea* gametophytes on agar from each species.

Table 1. Samples used in this study. Pressed vouchers are accessioned at IND and include more complete locality data. Samples are sexually reproducing taxa, except where noted.

Species	Origin of Sample
PHANEROPHLEBIA	
P. auriculata Underw.	ARIZONA: Cochise County; *Yatskievych 83-161*
P. juglandifolia (Humb. & Bonpl. ex Willd.) J. Smith (diploid)	MEXICO: Edo. Chiapas; *Yatskievych et al. 85-182*
P. juglandifolia (tetraploid)	COSTA RICA: Prov. San José *Yatskievych & McCrary 86-13*
P. macrosora (Baker) Underw.	COSTA RICA: Prov. Heredia; *Yatskievych & McCrary 86-30*
P. nobilis (Schlecht. & Cham.) C. Presl	MEXICO: Edo. México; *Yatskievych et al. 85-211*
P. pumila (Mart & Gal.) Fée	MEXICO: Edo Oaxaca; *Yatskievych et al. 85-139*
P. remotispora (Fourn.) Underw.	MEXICO: Edo. Hidalgo; *Yatskievych et al. 83-353*
P. umbonata Underw.	MEXICO: Edo. Nuevo León; *Yatskievych & Wollenweber 83-87*
CYRTOMIUM	
C. falcatum (Thunb. ex L. f.) C. Presl (apomictic)	SOUTH CAROLINA: Charleston County; *Yatskievych & McCrary 83-184*
C. fortunei J. Smith (apomictic)	SOUTH CAROLINA: Charleston County; *Yatskievych & McCrary 83-185*
C. macrophyllum (Makino) Tagawa (apomictic)	JAPAN: Honshu, Shiga Pref., *Mitsuta in 1984*
POLYSTICHUM	
P. acrostichoides (Michx.) Schott	INDIANA: Monroe County; *Yatskievych & McCrary 83-258*
P. imbricans (D. C. Eaton) D. H. Wagner ssp. *curtum* Ewan) D. H. Wagner	CALIFORNIA: Riverside County; *Yatskievych & McCrary 84-119*
P. lonchitis (L.) Roth	ARIZONA: Coconino County; *Yatskievych & WIndham 85-302*
P. munitum (Kaulf.) C. Presl	CALIFORNIA: Marin County; *Yatskievych & McCrary 84-131*
MISCELLANEOUS TAXA	
Pteridium aquilinum (L.) Kuhn var. *latiusculum* (Desv.) Heller	INDIANA: Monroe County; *Yatskievych & McCrary 83-259*
Onoclea sensibilis L.	INDIANA: Brown County; *Yatskievych & Johnson 84-178*

Results and Discussion

Spore germination and gametophyte development and morphology in the three genera under study are the typical types found in the family Dryopteridaceae (Nayar and Kaur, 1971). Gametophyte development in selected species of *Cyrtomium* and *Polystichum* was described by Chandra and Nayar (1970) and is in general agreement with observations made during this study. For all species in the three genera, mature gametophytes are cordate and copiously glandular with unicellular trichomes. The normal sequence of gametangial development for isolated gametophytes in the sexual taxa involves produc-

tion of archegonia soon after development of a meristematic notch (7–8 weeks after spore germination), at which point prothallia consist of ca. 100–150 cells. Antheridial production follows after 1–2 additional weeks of growth. For the three apomictic species (Table 1) no archegonial development was observed in any of our cultures and antheridial formation was both tardy and irregular.

Susceptibility to antheridiogen A and production of a native antheridiogen for all samples are reported in Table 2. All controls performed as expected. Reduced growth of *Onoclea* gametophytes on 50% *Pteridium* agar, as had been noted by Haufler and Gastony (1978), was not observed during this study, probably due to slightly differing concentrations of antheridiogen and other solutes in the agar stocks. For the sexual taxa analyzed, all samples responding to antheridiogen A also produced a native antheridiogen. That these native compounds correspond to the antheridiogen A type is demonstrated by the fact that gametophytes of *Onoclea* responded to them in all cases. For *Phanerophlebia nobilis* the response of sample gametophytes to both native antheridiogen and antheridiogen A from *Pteridium* was slight and was indicated only by the slightly precocious production of greater numbers of antheridia in comparisons of sample and control plates. Previous studies have documented infra- and interpopulational variation in levels of antheridiogen sensitivity for various species (Schneller et al., 1990). Thus, further tests to determine whether antheridiogen susceptibility varies between populations of this and other *Phanerophlebia* species would be instructive.

The present study confirms previous findings (Voeller, 1964) that *Polystichum acrostichoides* responds to antheridiogen A, whereas the apomictic *Cyrtomium falcatum* does not. Gametophytes of *C. fortunei* and *C. macrophyllum*, however, were found to produce a native antheridiogen (Table 2), as shown by the response of *Onoclea* gametophytes when grown on agar from these species. However, neither of these taxa showed any response to antheridiogen A, nor did either respond to its own native antheridiogen (gametophytes on treatment agar showed growth rates and unresponsive, cordate morphologies identical to those grown simultaneously on unenriched agar). This unusual observation presumably relates to the apomictic life cycles of these taxa and correlates with the observed irregular production of antheridia by prothallia of these plants. It is not, however, the only type of antheridiogen response possible for apomictic taxa. For the apogamous *Bommeria pedata* (Sw.) Fourn., Haufler and Gastony (1978) showed normal levels of archegonial and antheridial production and strong response to antheridiogen A, in spite of the redundancy of such a pherormonal response in an apomictic plant. If the inference of these authors is correct, that *B. pedata* is a recently evolved apomict because it retains its antheridiogen susceptibility, then the data for the *Cyrtomium* species suggest that they are of less recent origin, because they *have* lost their antheridiogen susceptibility, but continue to produce a native compound.

As outlined by Willson (1981), production of an antheridiogen also might convey a potential selective advantage for such taxa through a kind of allelopathic mechanism. Although the pheremone would have no effect on development of neighboring gametophytes of the same species (which are not susceptible), it would inhibit growth and production of archegonia in prothallia of adjacently growing gametophytes of other fern species that do respond to an antheridiogen. These gametophytes presumably would compete with the *Cyrtomium* gametophytes for space and nutrients. Such inference, however, remains purely speculative at this point.

Table 2. A summary of tests for response of sample taxa to two concentrations of antheridiogens (see text for details).

species	*response to Antheridiogen A* 50%	25%	*production of native antheridiogen* 50%	25%
PHANEROPHLEBIA				
P. auriculata	-	-	-	-
P. juglandifolia (2x)	-	-	-	-
P. juglandifolia (4x)	-	-	-	-
P. macrosora	-	-	-	-
P. nobilis	+	+	+	+
P. pumila	-	-	-	-
P. remotispora	+	+	+	+
P. umbonata	+	+	+	+
CYRTOMIUM				
C. falcatum	-	-	-	-
C. fortunei	-	-	+	+
C. macrophyllum	-	-	+	+
POLYSTICHUM				
P. acrostichoides	+	+	+	+
P. imbricans ssp. *curtum*	-	-	-	-
P. lonchitis	-	-	-	-
P. munitum	-	-	-	-

Antheridiogen response appears to be a poor character for defining natural lineages within the polystichoid ferns, since reaction to these compounds is sporadic among species in various groups in this assemblage. Within *Planerophlebia,* however, the species exhibiting susceptibility to antheridiogen A are quite closely related. Recent studies comparing the chloroplast genomes of *Phanerophlebia* species though analysis of restriction site mutations have provided evidence that *P. nobilis* and *P. remotispora* are probably best treated as conspecific varieties of *P. nobilis* (Yatskievych et al., 1988; Yatskievych, 1990). These studies also have shown that *P. nobilis* is a potential diploid progenitor of allotetraploid *P. auriculata. Phanerophlebia umbonata* is probably the other progenitor of *P. auriculata,* based on morphological and biogeographic considerations (Yatskievych, 1990). Thus, the lack of antheridiogen response in tetraploid *P. auriculata* is puzzling, in light of the response of both presumed progenitor taxa.

In contrast to the recent work of Haufler and Soltis (1984) on *Bommeria* gametophytes, the present laboratory findings do not correlate with field-based research on breeding systems of *Phanerophlebia* species. Allelic frequencies for the five diploid species compiled during a recent study of allozyme polymorphisms (Yatskievych, 1990) were subjected to analysis of inbreeding rates using techniques and a computer program developed by Kent Holsinger (1987). The maximum likelihood and bootstrap estimates and confidence intervals are summarized in Table 3. There is no significant difference in inbreeding rates between the antheridiogen producing taxa and those that neither produced nor responded to an antheridiogen. All species are strongly outcrossing. Therefore, at least in the genus *Phanerophlebia,* antheridiogens are not the primary factor in determination of

Table 3. Intragametophytic selfing rates for five diploid species of *Phanerophlebia* calculated from isozyme data (Yatskievych, 1990), using maximum likelihood and bootstrap estimates (Holsinger, 1987). Values in parentheses are 95% confidence intervals.

Species	Antheridiogen Production	Maximum Likelihood Estimates	Bootstrap Estimates
P. juglandifolia	-	0.0919 (0.0000-0.2549)	0.1127 (0.0000-0.2476)
P. macrosora	-	0.0108 (0.0000-0.3365)	0.0000 (0.0000-0.2481)
P. nobilis	+	0.0000 (0.0000-0.1690)	0.0000 (0.0000-0.1559)
P. remotispora	+	0.0000 (0.0000-0.1491)	0.0000 (0.0000-0.1280)
P. umbonata	+	0.0000 (0.0000-0.2411)	0.0000 (0.0000-0.1870)

the breeding system. Whether antheridiogens are important in intergametophytic competition for space and resources (see discussion above) remains to be established by future research.

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